

1/7

Figure 1

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	10 20 30 40 50
Hordeum vulgare	---MASD-HR RFVLGSAVLL SVLAVAAATL E----- SVKDECQLGV
Oryza sativa	---MASN-KV VFSVLLLVV SVLAATATMA EYHHQDQVVY TPGPLCQPGM
Hordeum spontaneum	---MAFK--Y QLLLSAAVML AILAATVT-- ----- SFGDMCAPGD
Eleusine coracana	----- ----- ----- SVGTSCIPGM
Secale cereale	----- ----- ----- SVGGQCVPGI
Triticum durum	---MACKSSC SLLLLAHVLL SVLAA--A-- ----- SASGSCVPGV
Zea mays	MASSSSSSHR RLILAAAVLL SVLAAASA-- ----- SAGTSCVPGW
Triticum aestivum	---MASN-HR RFLLSGAVLL SVLAAVAA-L E----- SVEDECQPGV

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	60 70 80 90 100
Hordeum vulgare	DFPHNPLATC HTYVIKRVCG ---RGPSRPM LV----- -KERC
Oryza sativa	GYPMYPLPRC RALVKRQCVG ---RGTAATA EQ----- -VRRDC
Hordeum spontaneum	ALPANPLRAC RTYVVSQIC- --HVGPRLLST WD----- -MKRRC
Eleusine coracana	AIPHNPIDSC RWYVAKRACG ---VGPRLAT QE----- -MKARC
Secale cereale	AMPHNPGLGAC RTYVVSQIC- --HVGPRLLST WD----- -MKRRC
Triticum durum	AFRTNLLPHC RDYVLQQTG TFTPGLSKLP WMTSASIYSP GKPYLAKLYC
Zea mays	AIPHNPPLSC RWYVTSRTG ---IGPRLPW PE----- -LKRRC
Triticum aestivum	AFPHNALATC HTYVIKRVCG ---RGPSRPM LV----- -KERC

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	110 120 130 140 150
Hordeum vulgare	CRELAAMP-D HCRCEALRIL MDGVRTPE-- ----GRVVEG RLGDRRDCPR
Oryza sativa	CRQLAAVDDS WCRCEAISHM LGGIYRELG- ---APDVGHP MSEVFRGCRR
Hordeum spontaneum	CDELSAIP-A YCRCEALRII MDGTVTWQ-- ----GVFG-A YFKDMPNCPR
Eleusine coracana	CRQLEAIP-A YCRCEAVRIL MDGVVTP-- --SGQHEGR LLQDLPGCPR
Secale cereale	CDELLAIP-A YCRCEALRIL MDGVVTQ-- ----GVFEGG YLKDMPNCPR
Triticum durum	CQELAEIS-Q QCRCEALRYF IALPVPSQPV DPRSGNVGES GLIDLPGCPR
Zea mays	CRELADIP-A YCRCTALSIL MDGAIPPGP- ---DAQLEGR -LEDLPGCPR
Triticum aestivum	CRELAVVP-D YCRCEALRVL MDGVRAEE-- ----GHVVEG RLGDRRDCPR

	..... ..... ..... ..... ..... ..... ..... ..... ..... .....
	160 170 180 190
Hordeum vulgare	EEQRAFAATL VTAAECNLSS VQAPGVRLVL LADG-----
Oryza sativa	GDLERAAASL PAFCNVDIPN GGG-GVCYWL ARSGY-----
Hordeum spontaneum	VMQTSYAANL VNPQECNLWT IHGSPSCPEL QPGYEVVL--
Eleusine coracana	QVQRAFAPKL VTEVECNLAT IHGGPFCLSL LGAGE-----
Secale cereale	VTQSYAATL VAPQECNLPT IHGSPYCPTL QAGY-----
Triticum durum	EMQWDFVRLI VAPGQCNLAT IHNVRYPV EQPLWI----
Zea mays	EVQRGFAATL VTEAECNLAT ISGVAECPWI LGGGTTPSK--
Triticum aestivum	EAQREFAATL VTAAECNLPT VS--GVGSTL GATGRWMTIE LPK

Figure 2

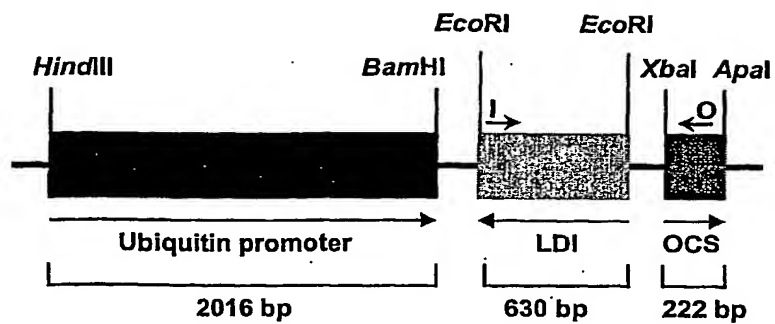
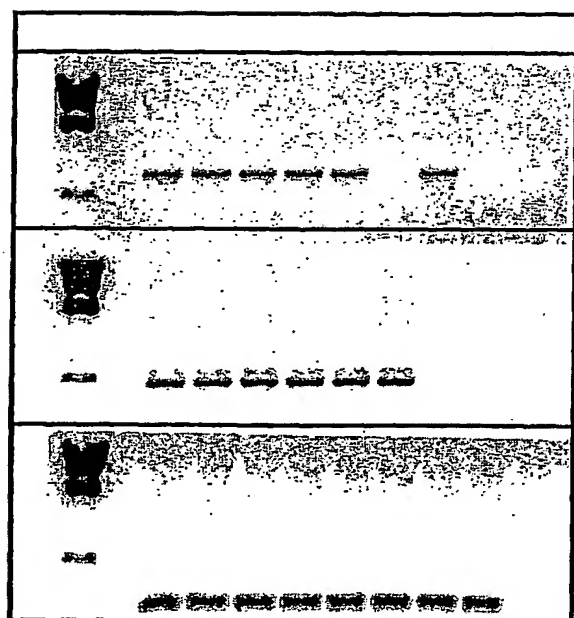


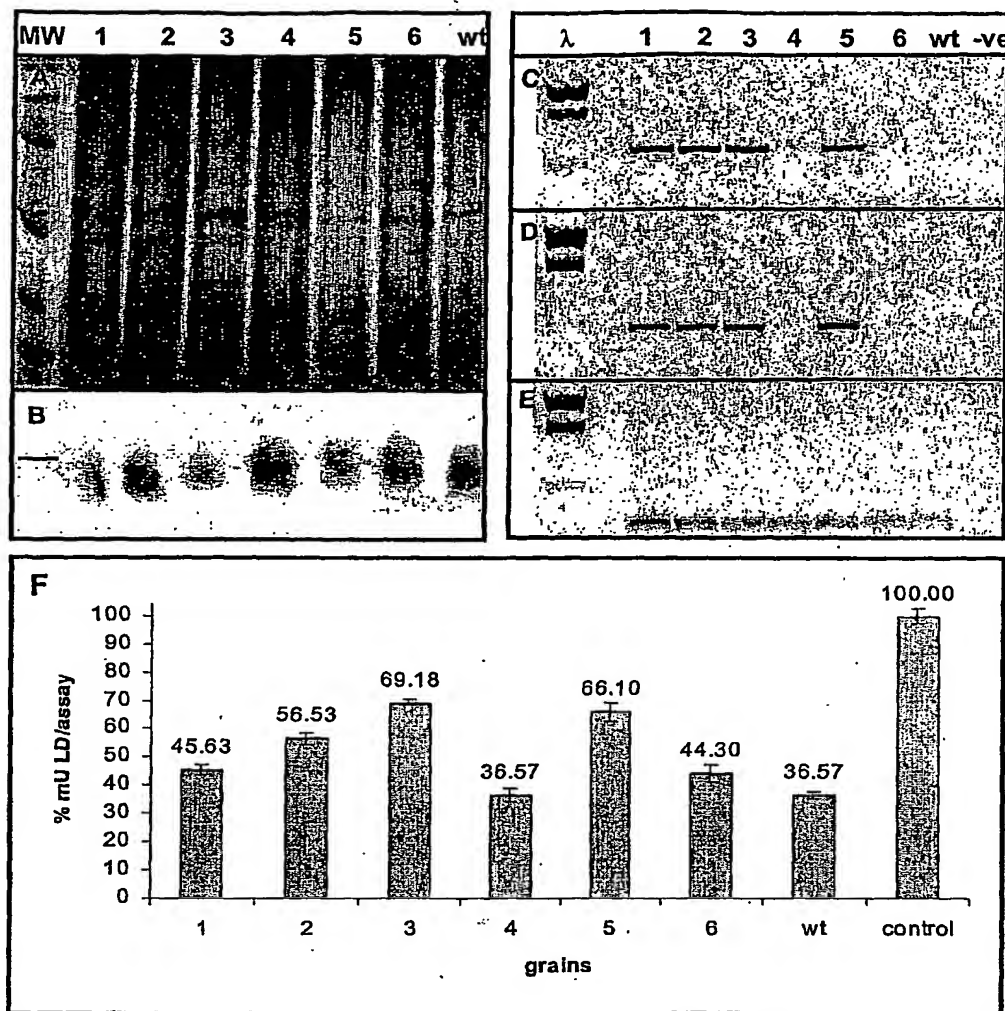
Figure 3



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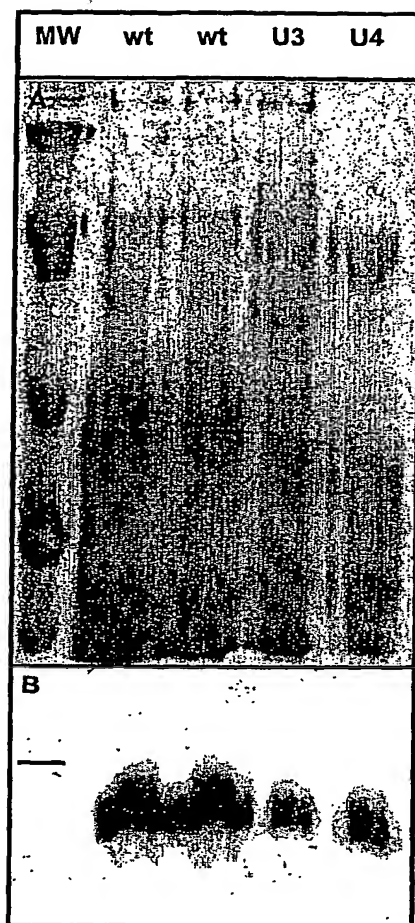
Figure 4



(A) 10% SDS-PAGE of LDI extracts corresponding to 5  $\mu$ g protein.  
 (B) Immunoblot developed with antiserum against LDI; bar represents 21.5 kD.  
 (C) PCR of genomic DNA with primers inhib-6 + OCS-II for the *LDI* gene in antisense direction (817 bp).  
 (D) PCR of genomic DNA primers BAR-I + BAR-II for the *bar* gene (534 bp).  
 (E) PCR of genomic DNA primers TUB-F + TUB-R for the *tubulin* gene (217 bp).  
 (F) LDI activity assay. The control represents the amount of LD used for each assay. LDI extracts corresponding to 10  $\mu$ g protein were mixed with LD and assayed for remaining LD activity. Each value represents the mean  $\pm$  SE of three replicate experiments.

1-6: six individual grains; wt: wildtype; MW: molecular weight marker sizes in kD are 97.4, 66.2, 45, 31, 21.5 and 14.4;  $\lambda$ :  $\lambda$ /Hind III molecular weight marker; -ve: negative control of PCR.

Figure 5



(A) 12% SDS-PAGE of LDI extracts corresponding to 5 $\mu$ g protein.

(B) Immunoblot developed with antiserum against LDI; bar represents 21.5 kD.

U3: homozygous T<sub>2</sub> generation transgenic line U3; U4: homozygous T<sub>2</sub> generation transgenic line U4; wt: wildtype; MW: molecular weight marker sizes in kD are 97.4, 66.2, 45, 31, 21.5 and 14.4.

Figure 6

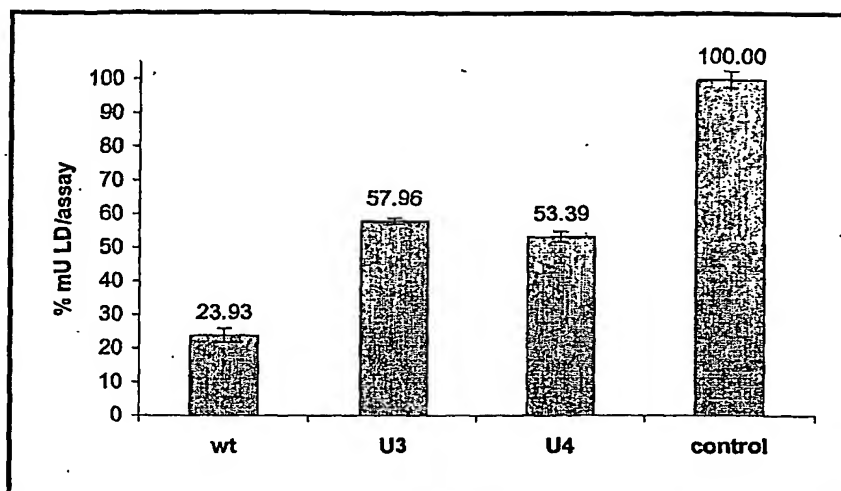
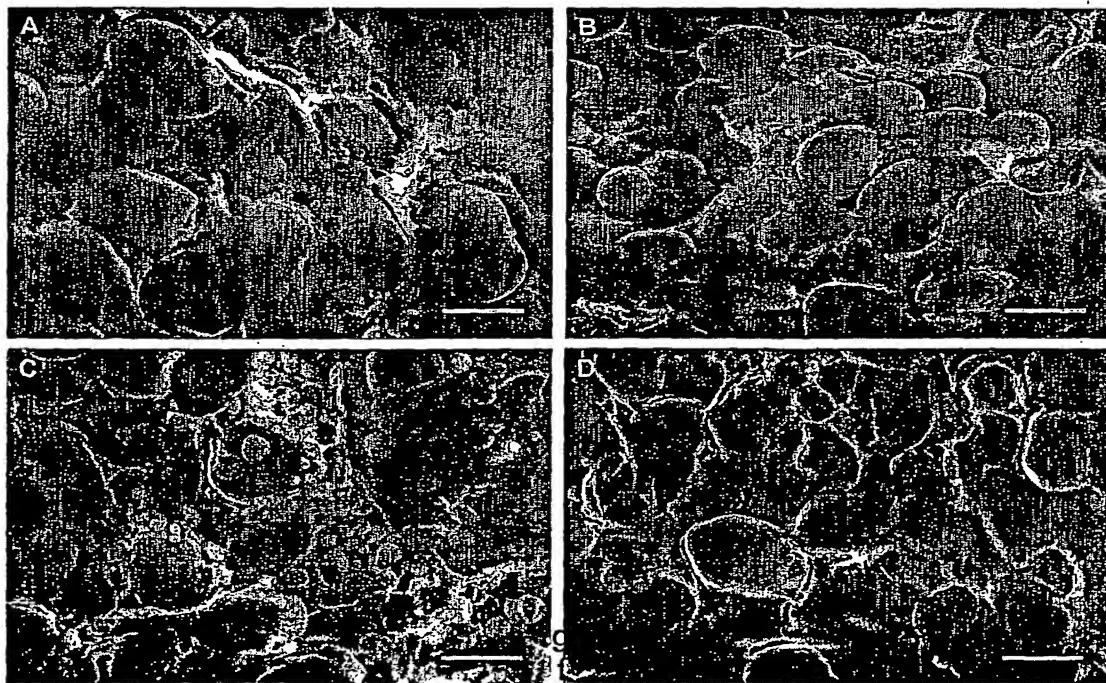


Figure 7



- (A) wildtype barley grain.  
(B) transgenic T<sub>2</sub> generation grain of homozygous line U4.  
(C) wildtype T<sub>1</sub> grain of heterozygous transgenic line U3.  
(D) transgenic T<sub>1</sub> grain of heterozygous line U3.  
Bar is 10  $\mu$ m long.

Figure 8

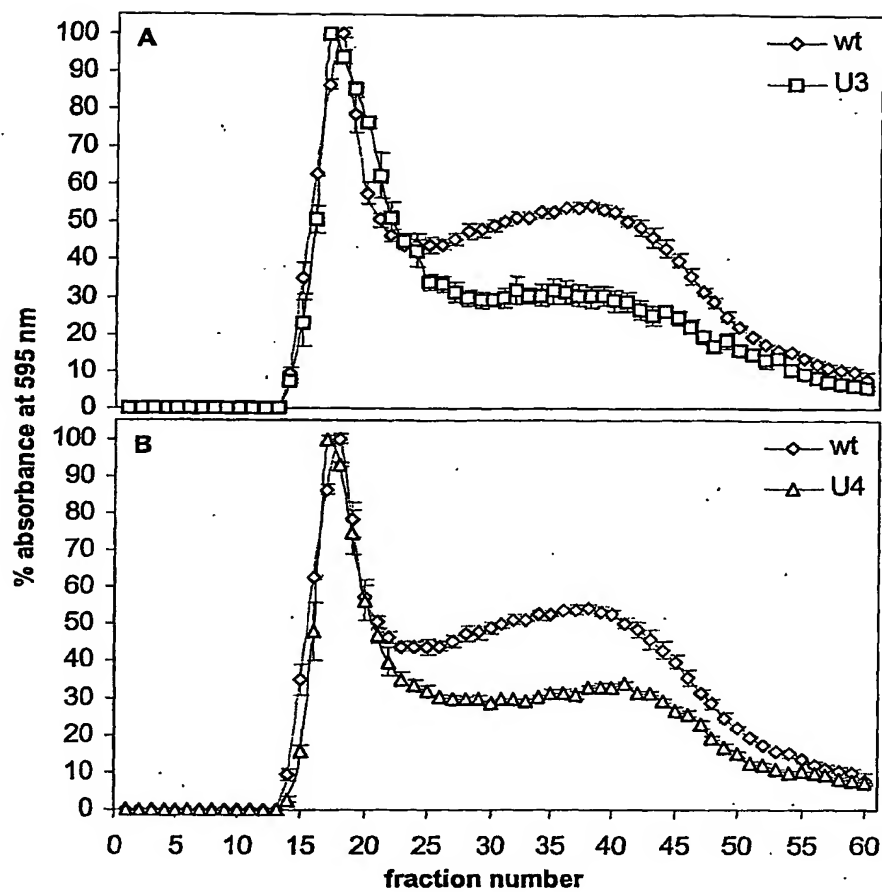


Figure 9

DNA % Identity	Hordeum vulgare SEQ. ID. No. 3	Hordeum vulgare SEQ. ID. No. 1	Triticum aestivum	Zea mays	Oryza sativa	Hordeum spontaneum
Hordeum vulgare SEQ. ID. No. 3	100	76.34				
Hordeum vulgare SEQ. ID. No. 1	98.84	100	77.27	63.45	42.35	43.09
Triticum aestivum			100	65.38	45.57	46.05
Zea mays				100	41.37	46.81
Oryza sativa					100	49.28
Hordeum spontaneum						100

% amino acid identities	Hordeum vulgare SEQ. ID. No. 4	Hordeum vulgare SEQ. ID. No. 2	Hordeum spontaneum	Oryza sativa	Triticum aestivum	Zea mays	Secale cereale	Eleusine coracana
Hordeum vulgare SEQ. ID. No. 4	100							
Hordeum vulgare SEQ. ID. No. 2	98.64	100	42.86	34.01	80.95	53.06	40.14	42.18
Hordeum spontaneum		43.54	100	29.92	43.54	48.98	66.67	46.26
Oryza sativa		33.75	25.63	100	31.88	33.13	23.75	25
Triticum aestivum		78.43	41.83	33.33	100	54.25	39.22	39.22
Zea mays		50.97	46.45	35.48	53.55	100	43.87	52.9
Secale cereale		48.36	79.51	29.51	50.82	55.74	100	62.3
Eleusine coracana		51.22	54.47	29.27	50.41	66.67	61.79	100